GCACCGCGCG AGCTTGGCTG CTTCTGGGGC CTGTGTGGCC CTGTGTGTCG GAAAGATGGA
GCAAGAAGCC GAGCCCGAGG GGCGGCCGCG ACCCCTCTGA CCGAGATCCT GCTGCTTTCG
CAGCCAGGAG CACCGTCCCT CCCCGGATTA GTGCGTACGA GCGCCCAGTG CCCTGGCCCG
GAGAGTGGAA TGATCCCCGA GGCCCAGGGC GTCGTGCTTC CGCAGTAGTC AGTCCCCGTG
AAGGAAACTG GGGAGTCTTG AGGGACCCCC GACTCCAAGC GCGAAAACCC CGGATGGTGA
GGAGCAGGCA AATGTGCAAT ACCAACATGT CTGTACCTAC TGATGGTGCT GTAACCACCT
CACAGATTCC AGCTTCGGAA CAAGAGACCC TGGTTAGACC AAAGCCATTG CTTTTGAAGT
TATTAAAGTC TGTTGGTGCA CAAAAAGACA CTTATACTAT GAAAGAGGTT CTTTTTTATC
TTGGCCAGTA TATTATGACT AAACGATTAT ATGATGAGAA GCAACAACAT ATTGTATATT
GTTCAAATGA TCTTCTAGGA GATTTGTTTG GCGTGCCAAG CTTCTCTGTG AAAGAGCACA
GGAAAATATA TACCATGATC TACAGGAACT TGGTAGTAGT CAATCAGCAG GAATCATCGG
ACTCAGGTAC ATCTGTGAGT GAGAACAGGT GTCACCTTGA AGGTGGGAGT GATCAAAAGG
ACCTTGTACA AGAGCTTCAG GAAGAGAAAC CTTCATCTTC ACATTTGGTT TCTAGACCAT
CTACCTCATC TAGAAGGAGA GCAATTAGTG AGACAGAAGA AAATTCAGAT GAATTATCTG
GTGAACGACA AAGAAAACGC CACAAATCTG ATAGTATTTC CCTTTCCTTT GATGAAAGCC
TGGCTCTGTG TGTAATAAGG GAGATATGTT GTGAAAGAAG CAGTAGCAGT GAATCTACAG
GGACGCCATC GAATCCGGAT CTTGATGCTG GTGTAAGTGA ACATTCAGGT GATTGGTTGG
ATCAGGATTC AGTTTCAGAT CAGTTTAGTG TAGAATTTGA AGTTGAATCT CTCGACTCAG
AAGATTATAG CCTTAGTGAA GAAGGACAAG AACTCTCAGA TGAAGATGAT GAGGTATATC
AAGTTACTGT GTATCAGGCA GGGGAGAGTG ATACAGATTC ATTTGAAGAA GATCCTGAAA
TTTCCTTAGC TGACTATTGG AAATGCACTT CATGCAATGA AATGAATCCC CCCCTTCCAT
CACATTGCAA CAGATGTTGG GCCCTTCGTG AGAATTGGCT TCCTGAAGAT AAAGGGAAAG
ATAAAGGGGA AATCTCTGAG AAAGCCAAAC TGGAAAACTC AACACAAGCT GAAGAGGGCT
TTGATGTTCC TGATTGTAAA AAAACTATAG TGAATGATTC CAGAGAGTCA TGTGTTGAGG
AAAATGATGA TAAAATTACA CAAGCTTCAC AATCACAAGA AAGTGAAGAC TATTCTCAGC
CATCAACTTC TAGTAGCATT ATTTATAGCA GCCAAGAAGA TGTGAAAGAG TTTGAAAGGG
AAGAAACCCA AGACAAAGAA GAGAGTGTGG AATCTAGTTT GCCCCTTAAT GCCATTGAAC
CTTGTGTGAT TTGTCAAGGT CGACCTAAAA ATGGTTGCAT TGTCCATGGC AAAACAGGAC
ATCTTATGGC CTGCTTTACA TGTGCAAAGA AGCTAAAGAA AAGGAATAAG CCCTGCCCAG
TATGTAGACA ACCAATTCAA ATGATTGTGC TAACTTATTT CCCCTAGTTG ACCTGTCTAT
AAGAGAATTA TATATTTCTA ACTATATAAC CCTAGGAATT TAGACAACCT GAAATTTATT
CACATATATC AAAGTGAGAA AATGCCTCAA TTCACATAGA TTTCTTCTCT TTAGTATAAT
TGACCTACTT TGGTAGTGGA ATAGTGAATA CTTACTATAA TTTGACTTGA ATATGTAGCT
CATCCTTTAC ACCAACTCCT AATTTTAAAT AATTTCTACT CTGTCTTAAA TGAGAAGTAC
TTGGTTTTTT TTTTCTTAAA TATGTATATG ACATTTAAAT GTAACTTATT ATTTTTTTTG
AGACCGAGTC TTGCTCTGTT ACCCAGGCTG GAGTGCAGTG GGTGATCTTG GCTCACTGCA
AGCTCTGCCC TCCCCGGGTT CGCACCATTC TCCTGCCTCA GCCTCCCAAT TAGCTTGGCC
TACAGTCATC TGCCACCACA CCTGGCTAAT TTTTTGTACT TTTAGTAGAG ACAGGGTTTC
ACCGTGTTAG CCAGGATGGT CTCGATCTCC TGACCTCGTG ATCCGCCCAC CTCGGCCTCC
CAAAGTGCTG GGATTACAGG CATGAGCCAC CG

FIG. 1A

GAGGAGCCGC CGCCTTCTCG TCGCTCGAGC TCTGGACGAC CATGGTCGCT CAGGCCCCGT
CCGCGGGGCC TCCGCGCTCC CCGTGAAGGG TCGGAAGATG CGCGGGAAGT AGCAGCCGTC
TGCTGGGCGA GCGGGAGACC GACCGGACAC CCCTGGGGGA CCCTCTCGGA TCACCGCGCT
TCTCCTGCGG CCTCCAGGCC AATGTGCAAT ACCAACATGT CTGTGTCTAC CGAGGGTGCT
GCAAGCACCT CACAGATTCC AGCTTCGGAA CAAGAGACTC TGGTTAGACC AAAACCATTG
CTTTTGAAGT TGTTAAAGTC CGTTGGAGCG CAAAACGACA CTTACACTAT GAAAGAGATT
ATATTTTATA TTGGCCAGTA TATTATGACT AAGAGGTTAT ATGACGAGAA GCAGCAGCAC
ATTGTGTATT GTTCAAATGA TCTCCTAGGA GATGTGTTTG GAGTCCCGAG TTTCTCTGTG
AAGGAGCACA GGAAAATATA TGCAATGATC TACAGAAATT TAGTGGCTGT AAGTCAGCAA
GACTCTGGCA CATCGCTGAG TGAGAGCAGA CGTCAGCCTG AAGGTGGGAG TGATCTGAAG
GATCCTTTGC AAGCGCCACC AGAAGAGAAA CCTTCATCTT CTGATTTAAT TTCTAGACTG
TCTACCTCAT CTAGAAGGAG ATCCATTAGT GAGACAGAAG AGAACACAGA TGAGCTACCT
GGGGAGCGGC ACCGGAAGCG CCGCAGGTCC CTGTCCTTTG ATCCGAGCCT GGGTCTGTGT
GAGCTGAGGG AGATGTGCAG CGGCGGCACG AGCAGCAGTA GCAGCAGCAG CAGCGAGTCC
ACAGAGACGC CCTCGCATCA GGATCTTGAC GATGGCGTAA GTGAGCATTC TGGTGATTGC
CTGGATCAGG ATTCAGTTTC TGATCAGTTT AGCGTGGAAT TTGAAGTTGA GTCTCTGGAC
TCGGAAGATT ACAGCCTGAG TGACGAAGGG CACGAGCTCT CAGATGAGGA TGATGAGGTC
TATCGGGTCA CAGTCTATCA GACAGGAGAA AGCGATACAG ACTCTTTTGA AGGAGATCCT
GAGATTTCCT TAGCTGACTA TTGGAAGTGT ACCTCATGCA ATGAAATGAA TCCTCCCCTT
CCATCACACT GCAAAAGATG CTGGACCCTT CGTGAGAACT GGCTTCCAGA CGATAAGGGG
AAAGATAAAG TGGAAATCTC TGAAAAAGCC AAACTGGAAA ACTCAGCTCA GGCAGAAGAA
GGCTTGGATG TGCCTGATGG CAAAAAGCTG ACAGAGAATG ATGCTAAAGA GCCATGTGCT
GAGGAGGACA GCGAGGAGAA GGCCGAACAG ACGCCCCTGT CCCAGGAGAG TGACGACTAT
TCCCAACCAT CGACTTCCAG CAGCATTGTT TATAGCAGCC AAGAAAGCGT GAAAGAGTTG
AAGGAGGAAA CGCAGCACAA AGACGAGAGT GTGGAATCTA GCTTCTCCCT GAATGCCATC
GAACCATGTG TGATCTGCCA GGGGCGGCCT AAAAATGGCT GCATTGTTCA CGGCAAGACT
GGACACCTCA TGTCATGTTT CACGTGTGCA AAGAAGCTAA AAAAAAGAAA CAAGCCCTGC
CCAGTGTGCA GACAGCCAAT CCAAATGATT GTGCTAAGTT ACTTCAACTA GCTGACCTGC
TCACAAAAAT AGAATTTTAT ATTTCTAACT

FIG. 1B

FIG. 2A

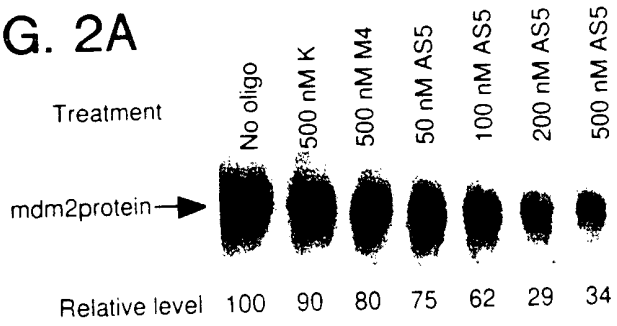


FIG. 2B

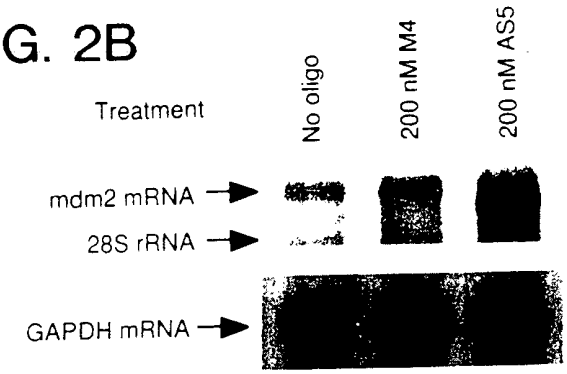
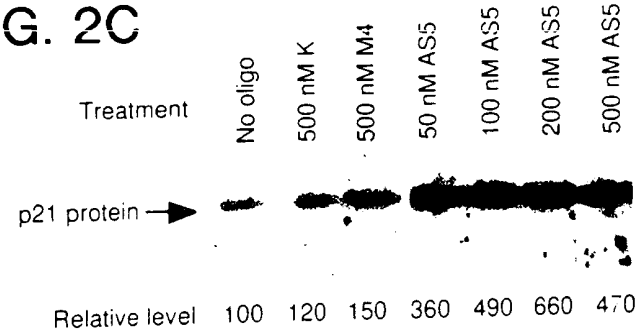


FIG. 2C



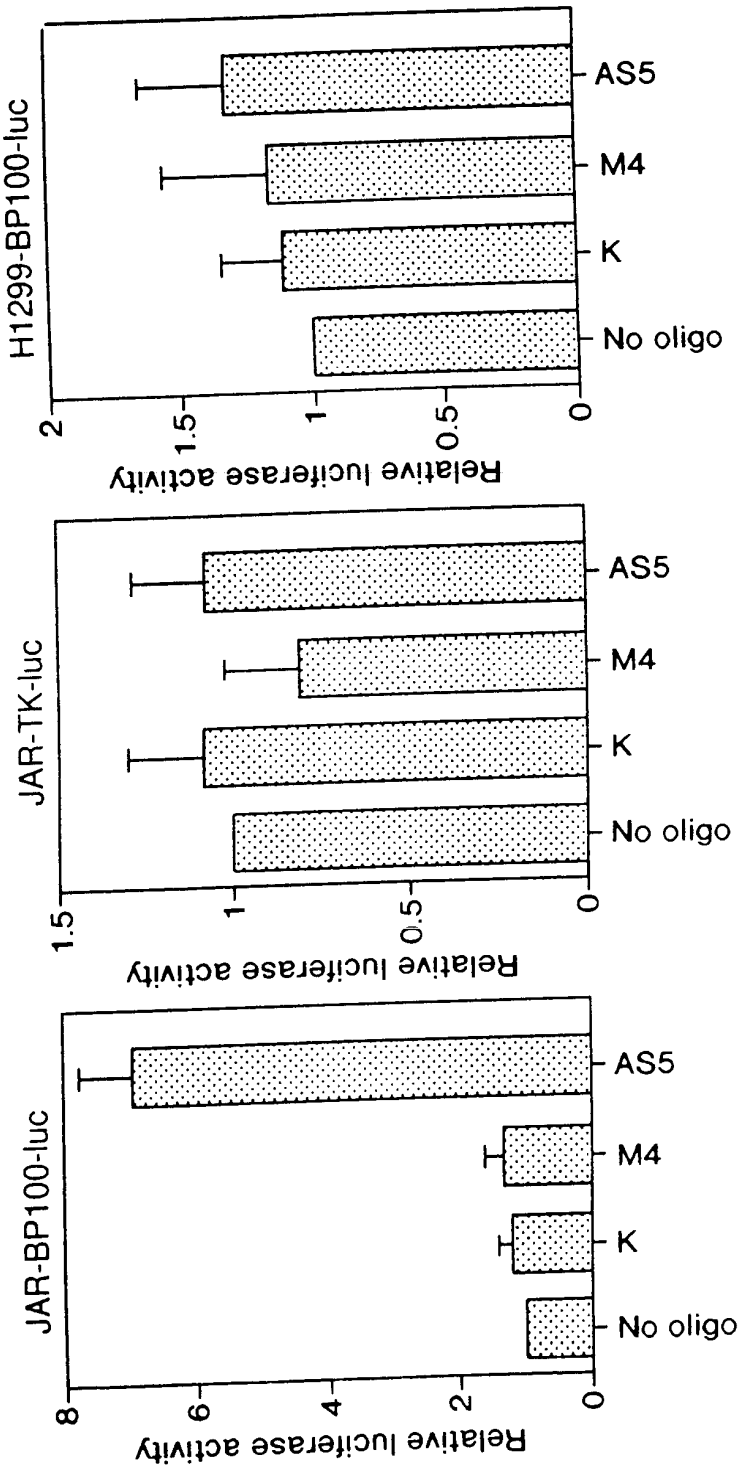


FIG. 3C

FIG. 3B

FIG. 3A

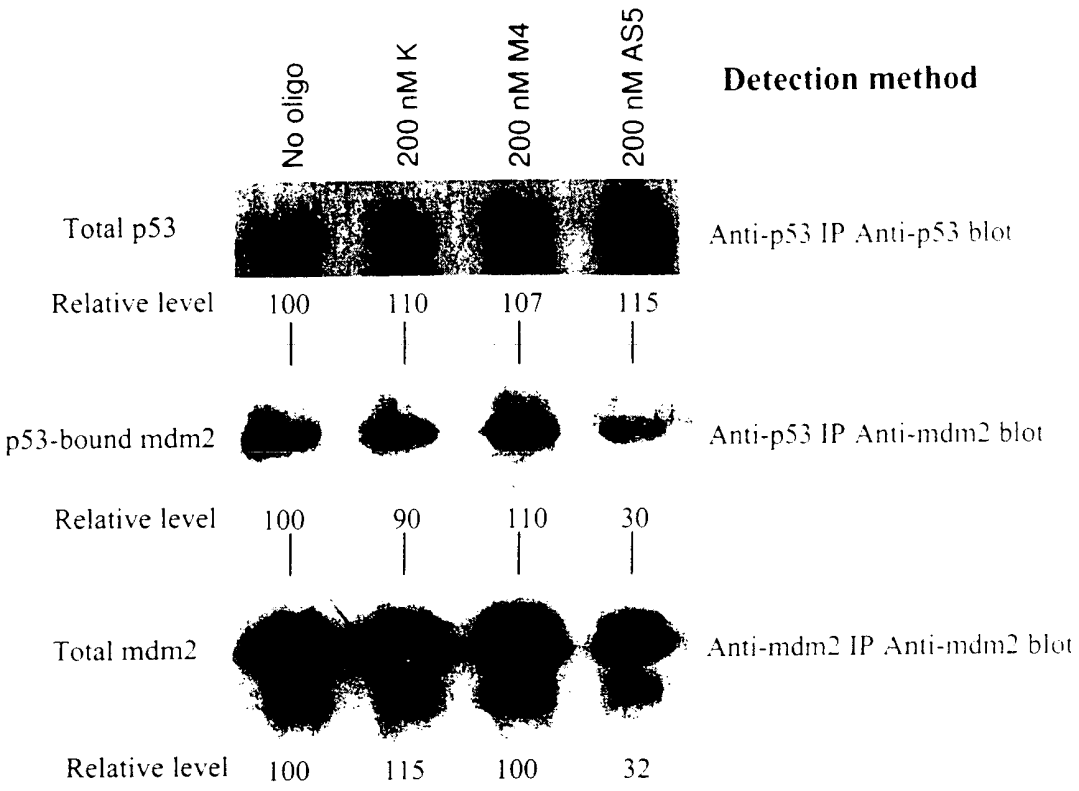

No oligo
200 nM K
200 nM M4
200 nM AS5
Detection method
Total p53
Anti-p53 IP Anti-p53 blot
Relative level
100
110
107
115
p53-bound mdm2
Anti-p53 IP Anti-mdm2 blot
Relative level
100
90
110
30
Total mdm2
Anti-mdm2 IP Anti-mdm2 blot
Relative level
100
115
100
32

FIG. 4

JAR + 200 nM AS5

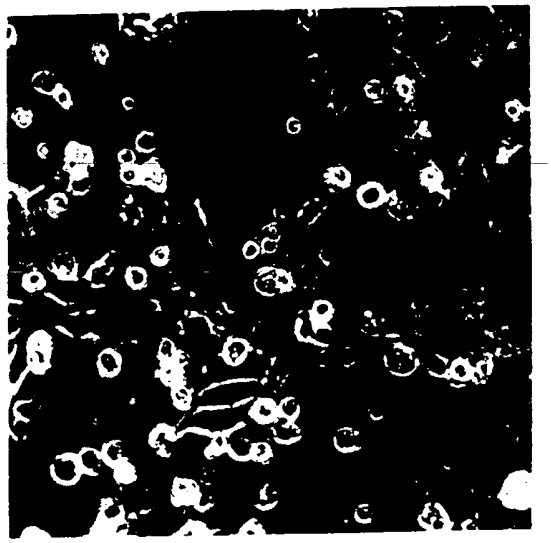


FIG. 5A

JAR + 200 nM M4

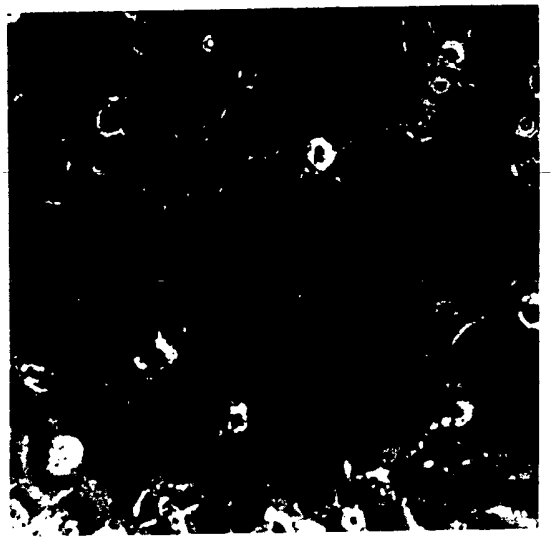


FIG. 5B

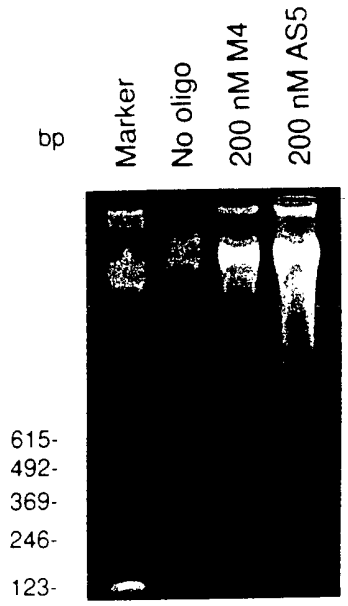


FIG. 6

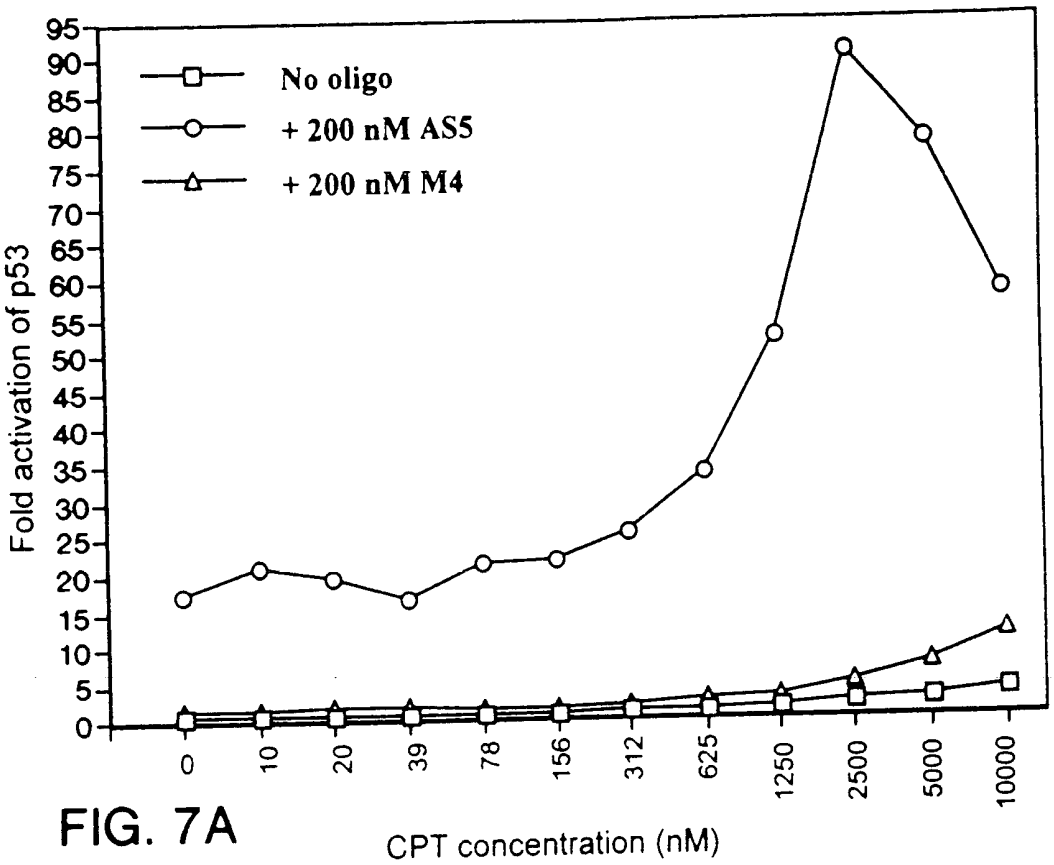

No oligo
+ 200 nM AS5
+ 200 nM M4
Fold activation of p53
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
0
10
20
39
78
156
312
625
1250
2500
5000
10000
CPT concentration (nM)

FIG. 7A

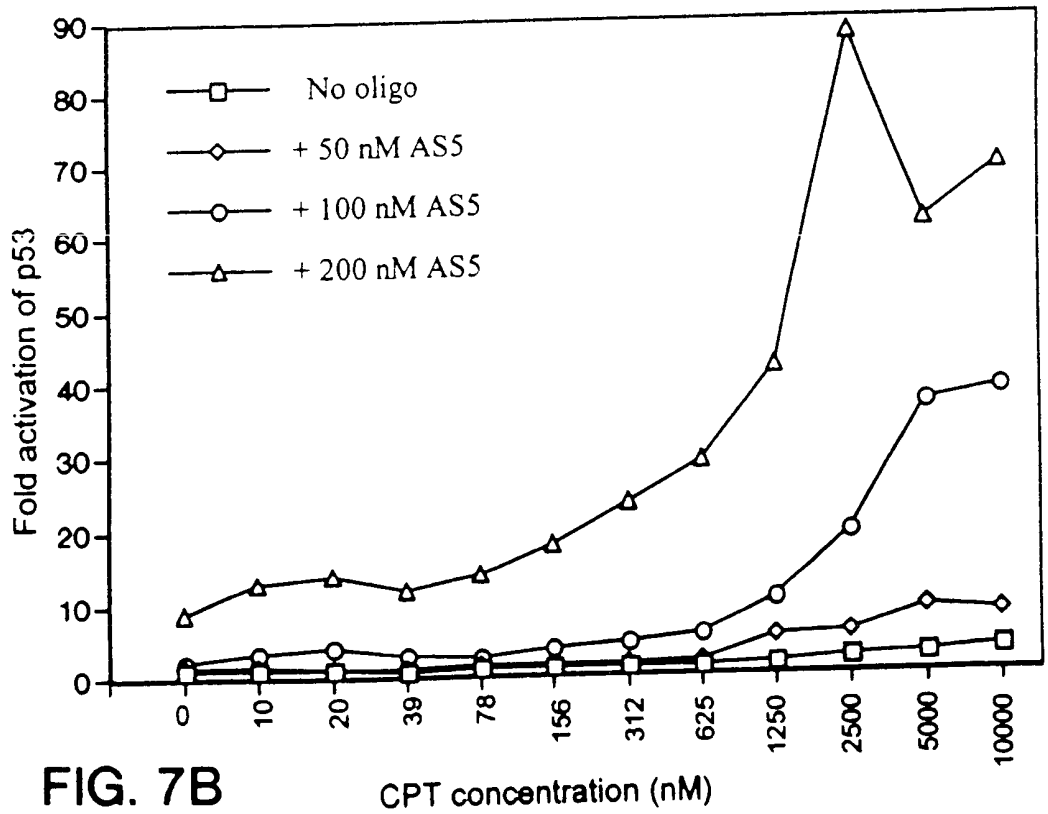

No oligo
+ 50 nM AS5
+ 100 nM AS5
+ 200 nM AS5
Fold activation of p53
90
80
70
60
50
40
30
20
10
0
0
10
20
39
78
156
312
625
1250
2500
5000
10000
CPT concentration (nM)

FIG. 7B

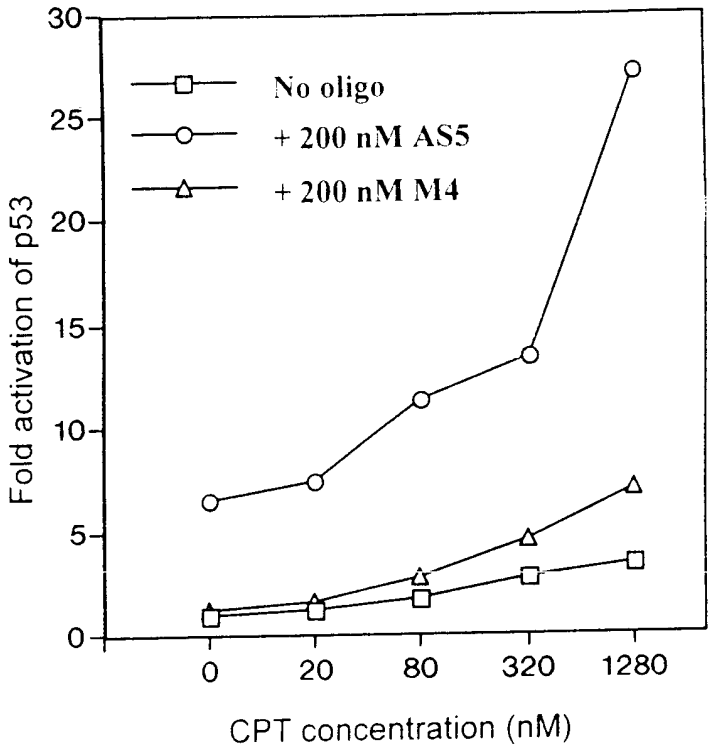

30
25
20
15
10
5
0
Fold activation of p53
No oligo
+ 200 nM AS5
+ 200 nM M4
0
20
80
320
1280
CPT concentration (nM)

FIG. 7C

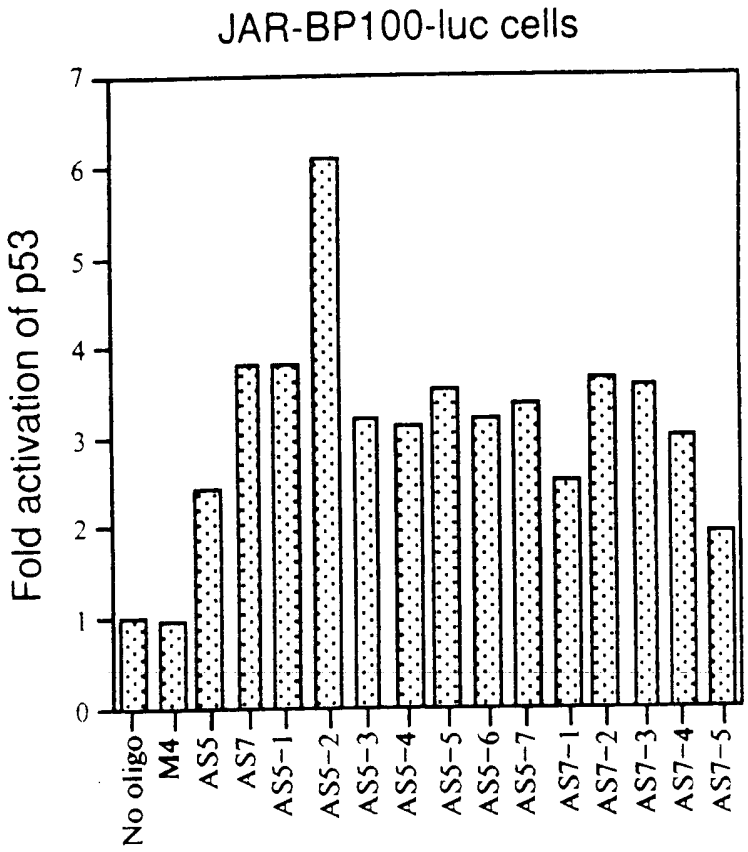

JAR-BP100-luc cells
Fold activation of p53
0
1
2
3
4
5
6
7
No oligo
M4
AS5
AS7
AS5-1
AS5-2
AS5-3
AS5-4
AS5-5
AS5-6
AS5-7
AS7-1
AS7-2
AS7-3
AS7-4
AS7-5

FIG. 8A

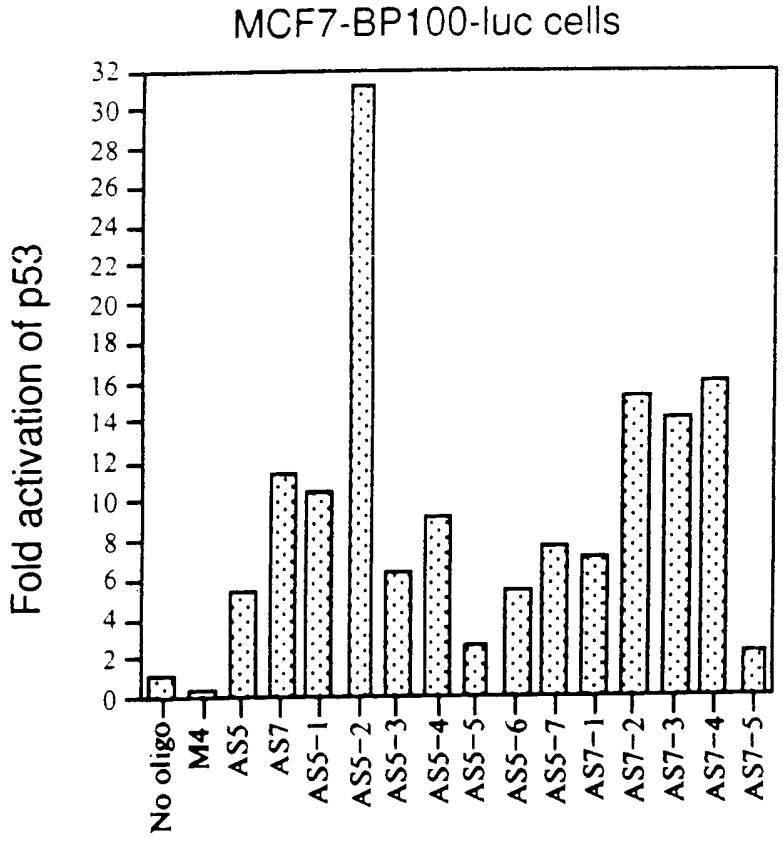

MCF7-BP100-luc cells
Fold activation of p53
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
No oligo
M4
AS5
AS7
AS5-1
AS5-2
AS5-3
AS5-4
AS5-5
AS5-6
AS5-7
AS7-1
AS7-2
AS7-3
AS7-4
AS7-5

FIG. 8B

353-TCCATGTAGACACTCACTCTTGTCCACAGTGGAACTTCCACCCTCACTAGTTTTCCTGGAACATGTTCTCGAA-425 SEQ ID NO: 49

SEQ ID NO.: 35 **AS5-1**: TGTAGACACTCACTCTTGTC **AS5**: GGAACTTCCACCCTCACTAG SEQ ID NO.: 28

SEQ ID NO.: 36 **AS5-2**: CACTCACTCTTGTCCACAGT **AS5-5**: ACCCTCACTAGTTTTCCTGG SEQ ID NO.: 39

SEQ ID NO.: 37 **AS5-3**: ACTCTTGTCCACAGTGGAAC **AS5-6**: CACTAGTTTTCCTGGAACAT SEQ ID NO.: 40

SEQ ID NO.: 38 **AS5-4**: TGTCCACAGTGGAACTTCCA **AS5-7**: TTCCTGGAACATGTTCTCGA SEQ ID NO.: 41

FIG. 9A

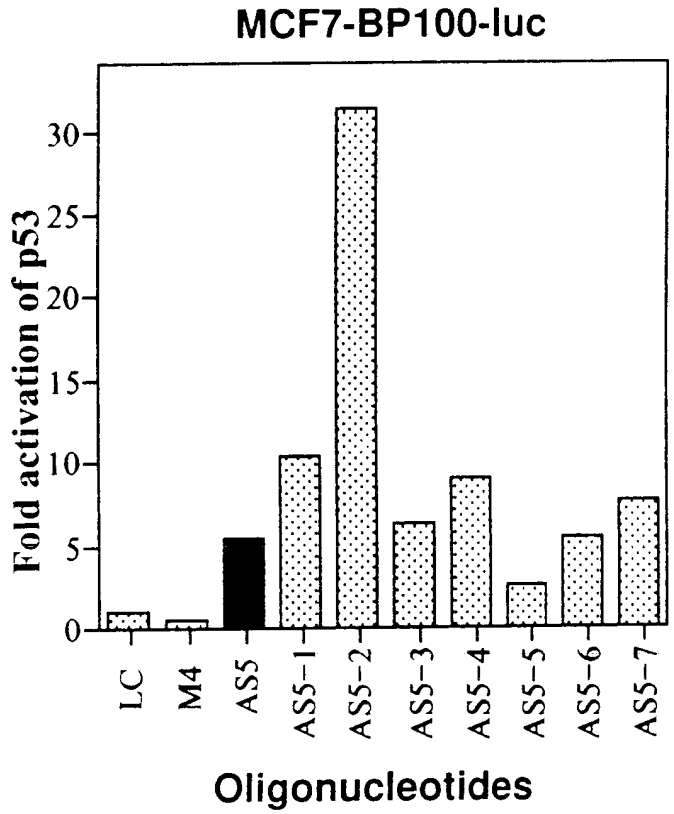

MCF7-BP100-luc
Fold activation of p53
0
5
10
15
20
25
30
LC
M4
AS5
AS5-1
AS5-2
AS5-3
AS5-4
AS5-5
AS5-6
AS5-7
Oligonucleotides

FIG. 9B

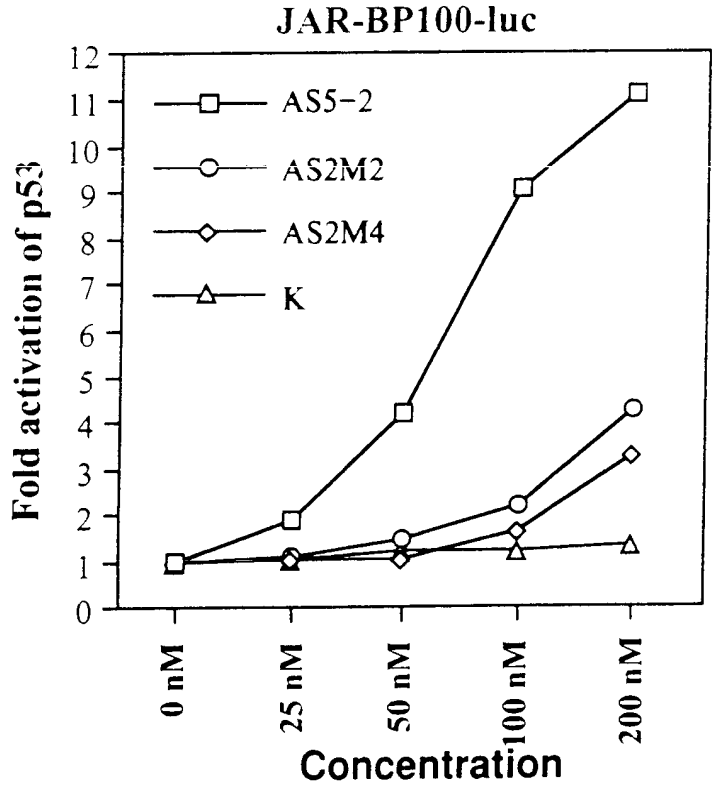

JAR-BP100-luc
Fold activation of p53
0
1
2
3
4
5
6
7
8
9
10
11
12
AS5-2
AS2M2
AS2M4
K
0 nM
25 nM
50 nM
100 nM
200 nM
Concentration

FIG. 9C

FIG. 10A-1 FIG. 10A-2

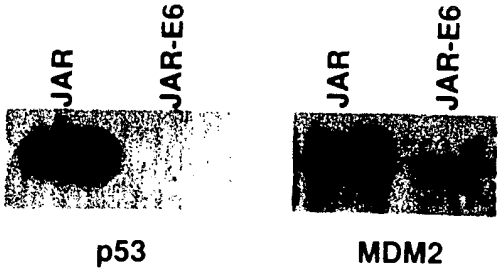


FIG. 10B-1 FIG. 10B-2

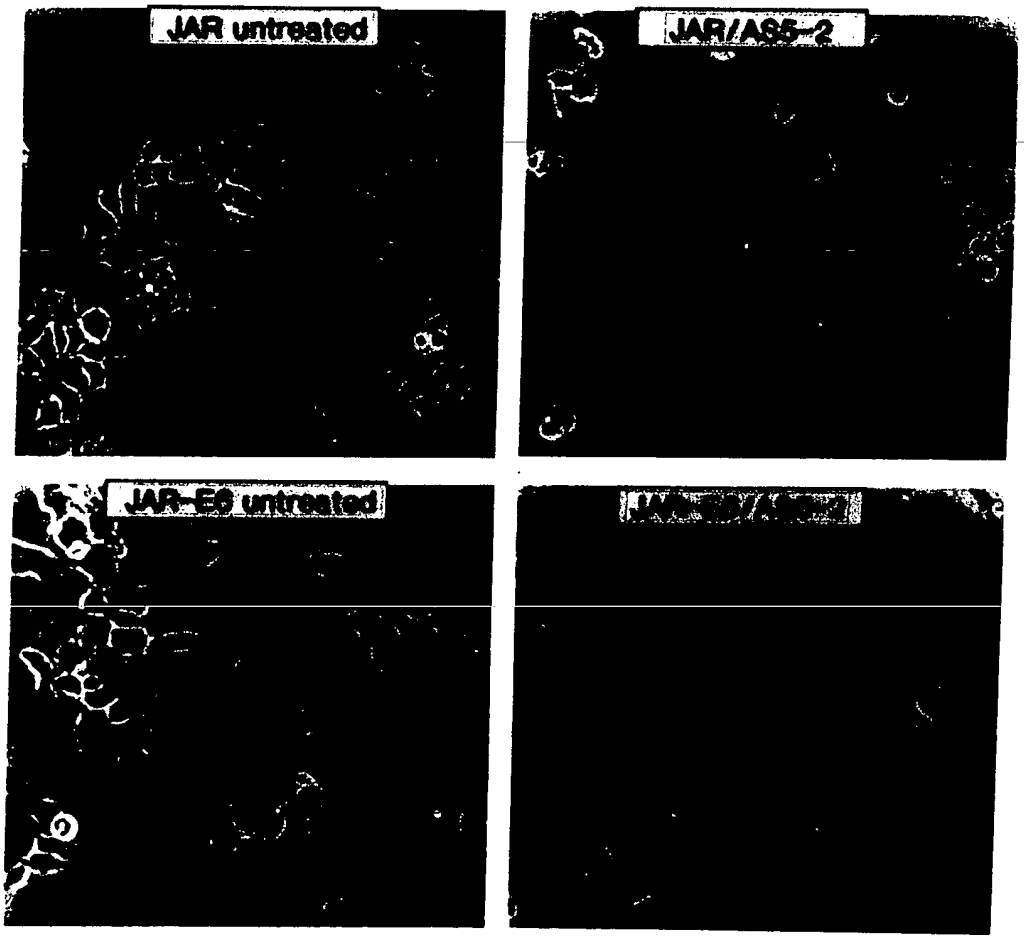


FIG. 10B-3 FIG. 10B-4

Control treated

AS5-2 treated

MCF-7
Breast tumor

FIG. 11A

FIG. 11B

SK-N-SH
Neuroblastoma

FIG. 11C

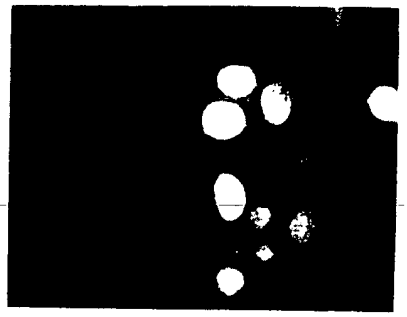


FIG. 11D

A172
Glioblastoma

FIG. 11E

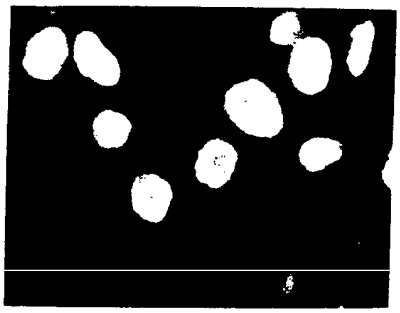


FIG. 11F

HT1080
Fibrosarcoma

FIG. 11G

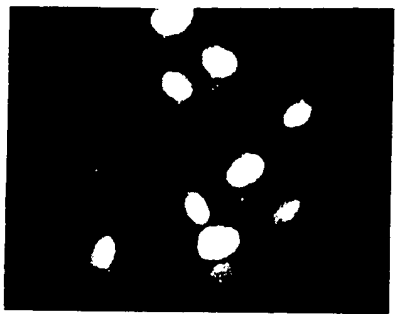


FIG. 11H

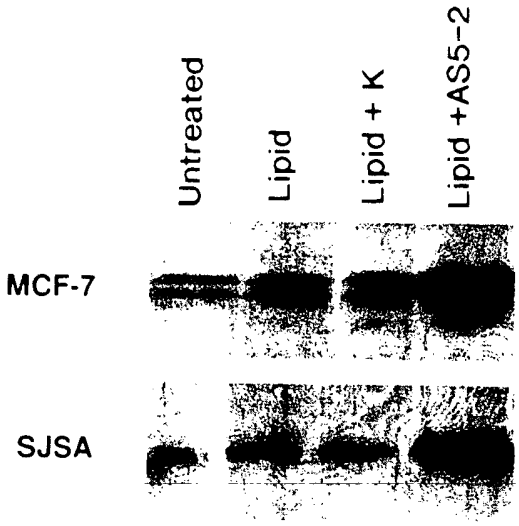


FIG. 12A

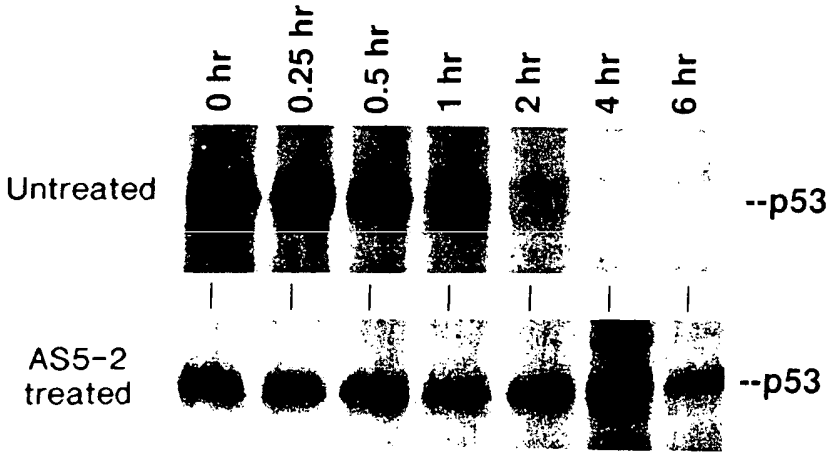


FIG. 12B

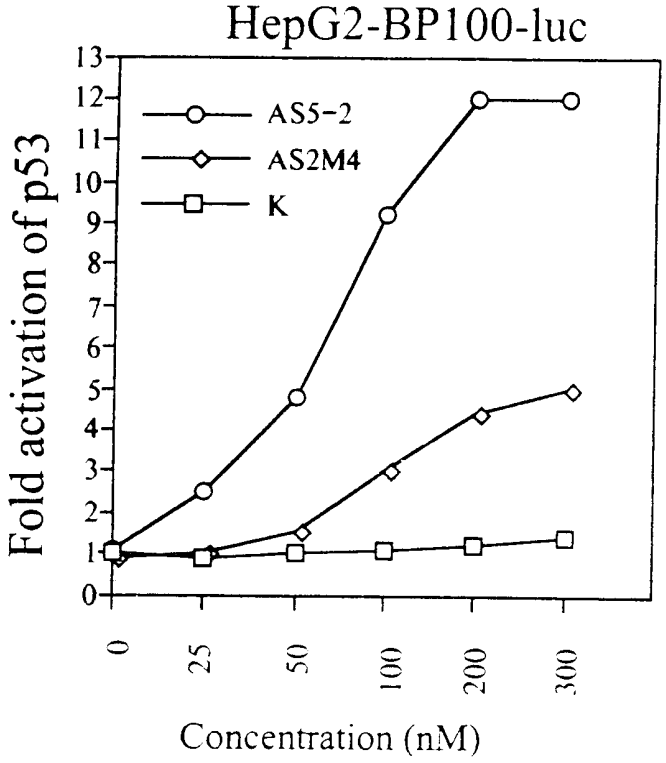


FIG. 13A

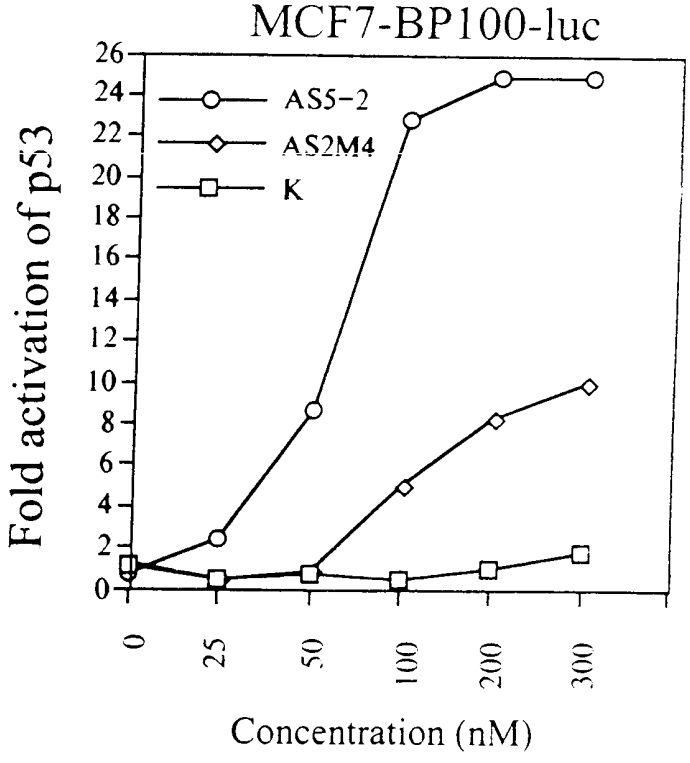


FIG. 13B

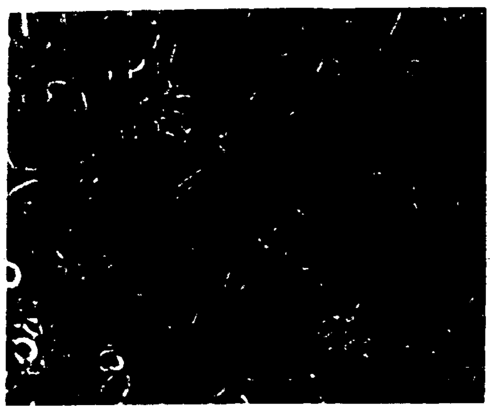
FIG. 14A

Control treated

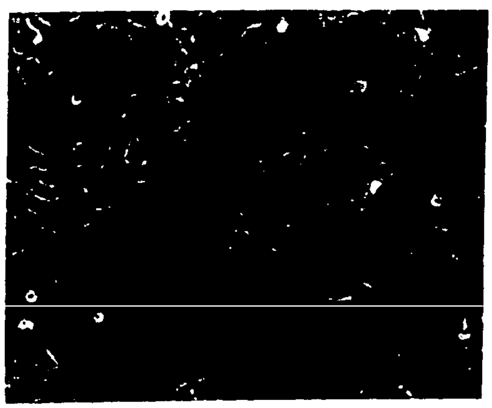
FIG. 14B

AS5-2 treated

HT1080



PA-1



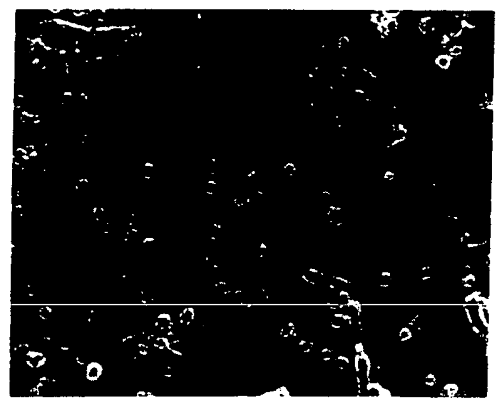


FIG. 14C

FIG. 14D

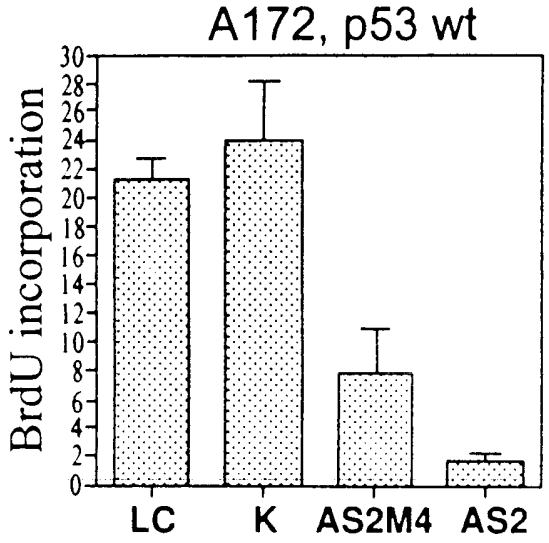


FIG. 15A

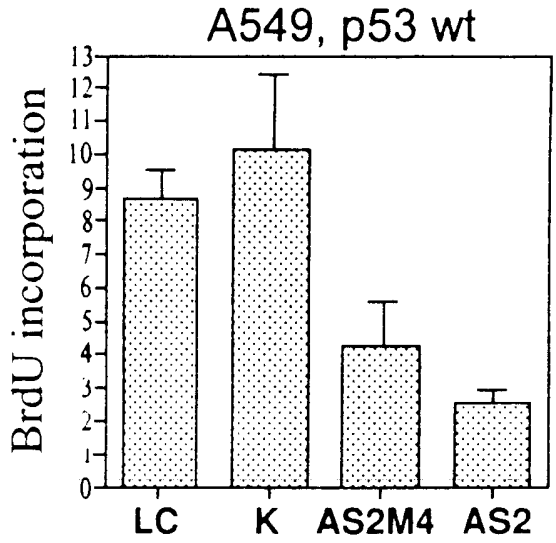


FIG. 15B

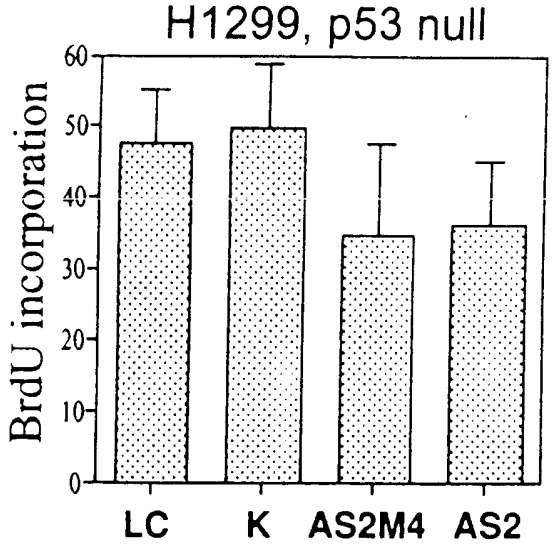


FIG. 15C

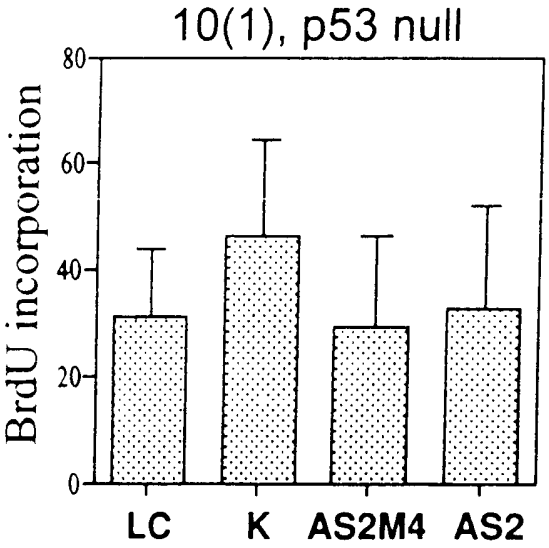


FIG. 15D

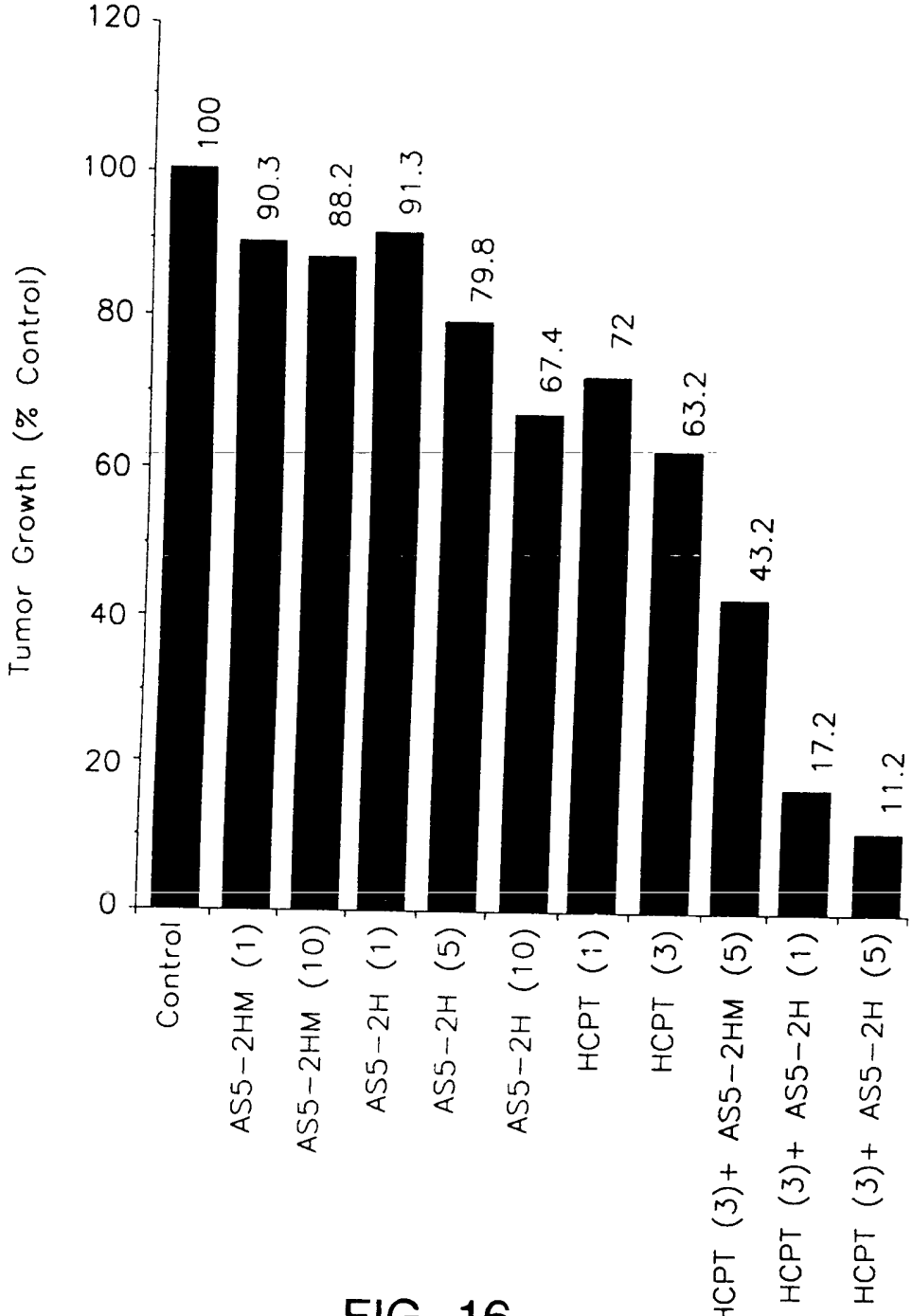

Tumor Growth (% Control)
120
100
80
60
40
20
0
100
90.3
88.2
91.3
79.8
67.4
72
63.2
43.2
17.2
11.2
Control
AS5-2HM (1)
AS5-2HM (10)
AS5-2H (1)
AS5-2H (5)
AS5-2H (10)
HCPT (1)
HCPT (3)
HCPT (3)+ AS5-2HM (5)
HCPT (3)+ AS5-2H (1)
HCPT (3)+ AS5-2H (5)

FIG. 16

Anticancer Activity of Anti-MDM2 Oligonucleotides

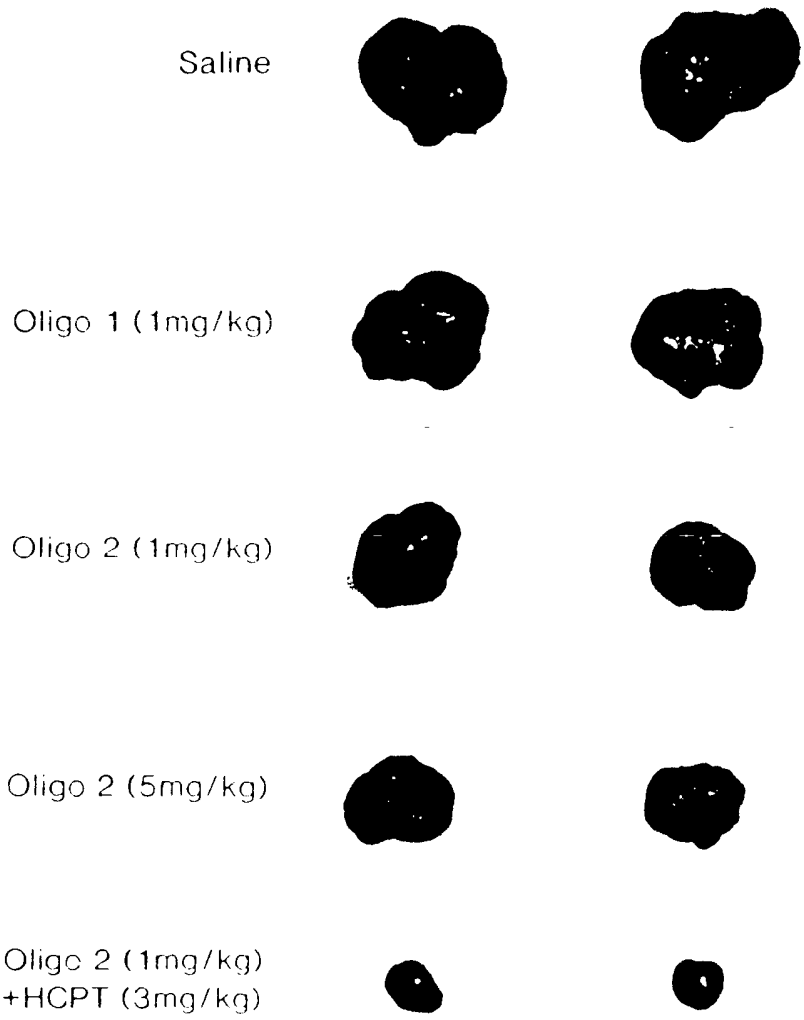


FIG. 17

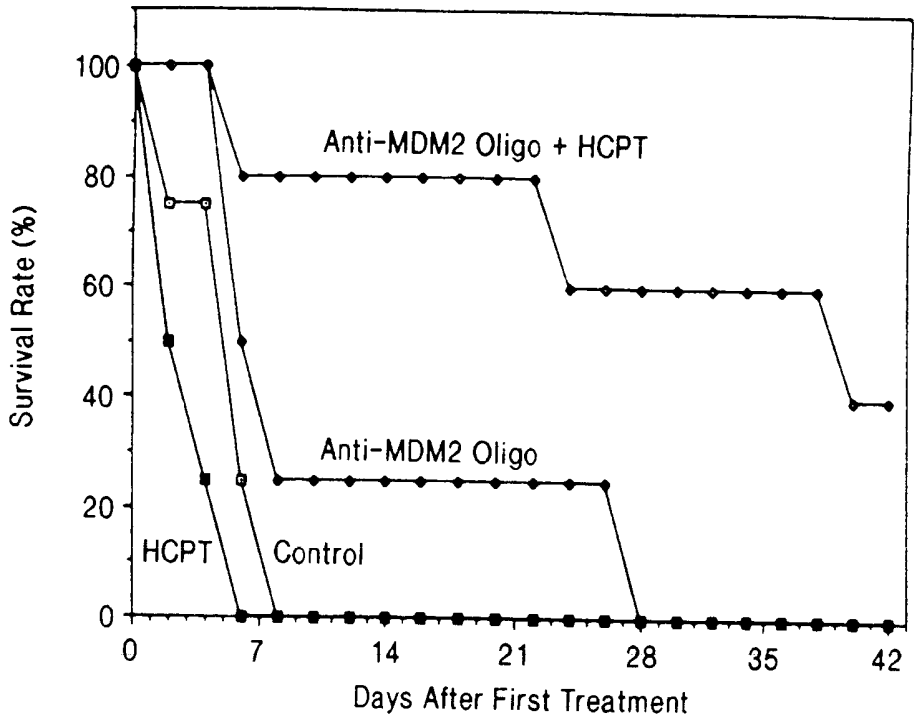


FIG. 18A

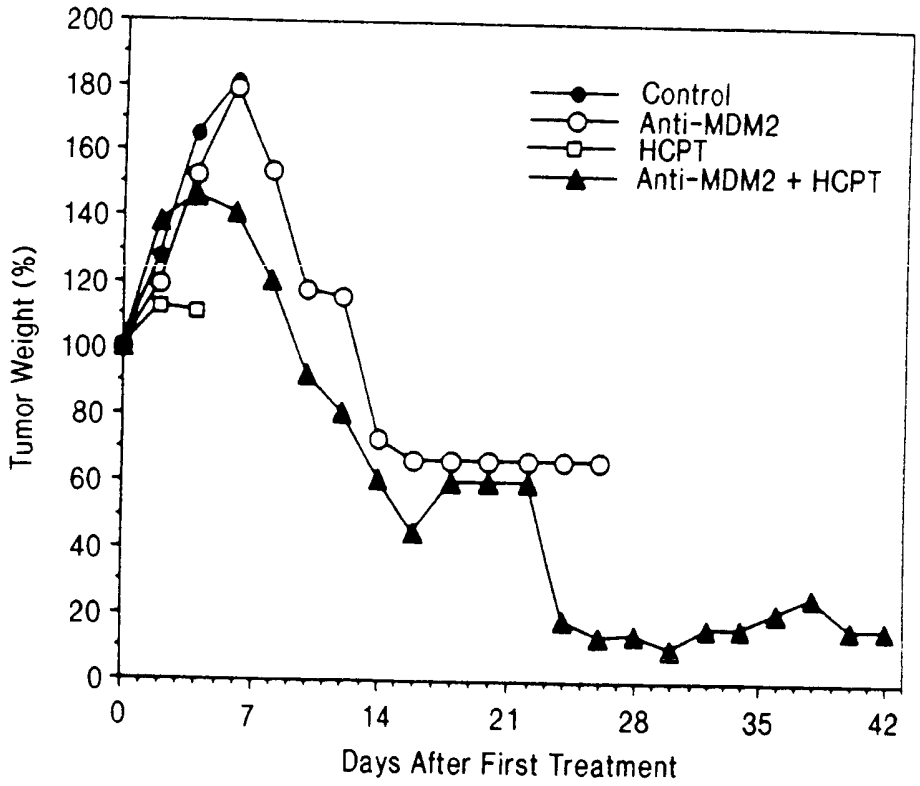


FIG. 18B

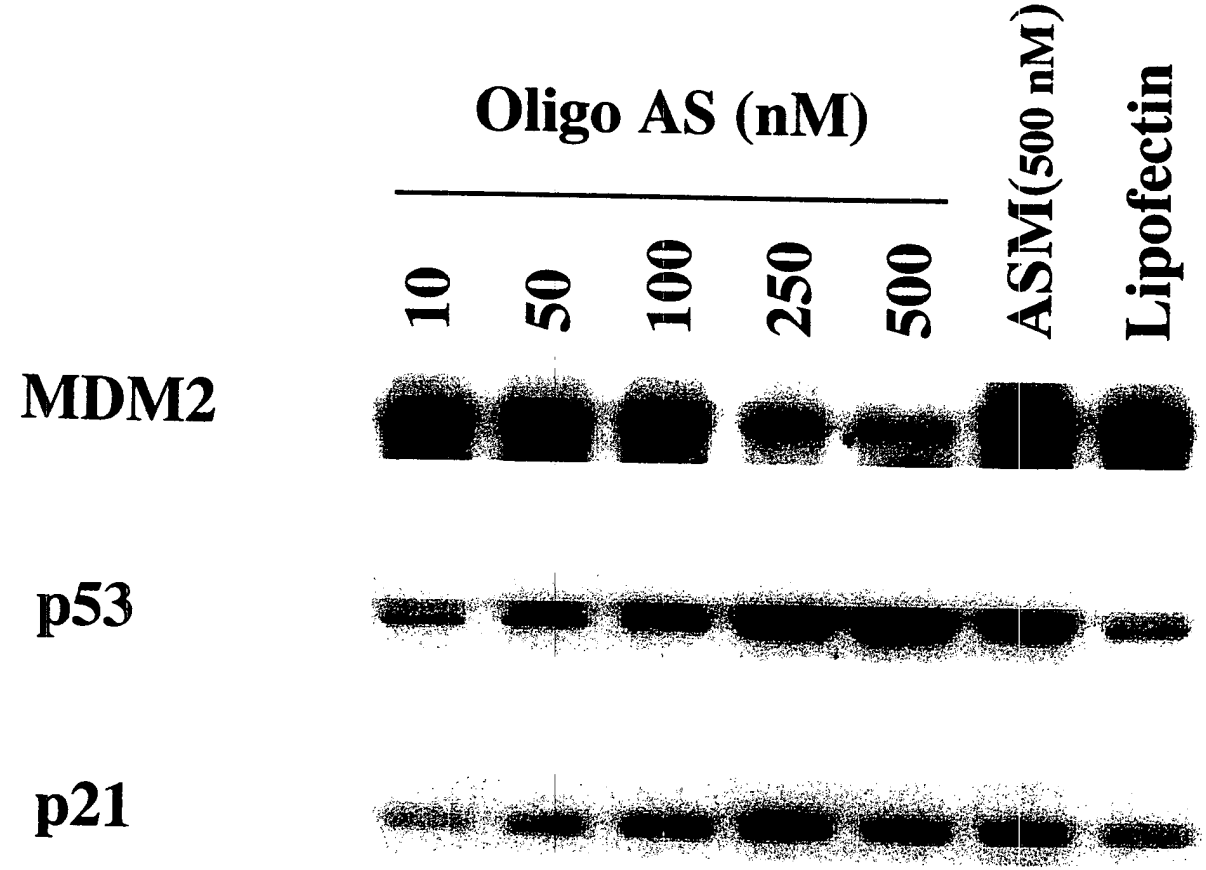


Fig. 19

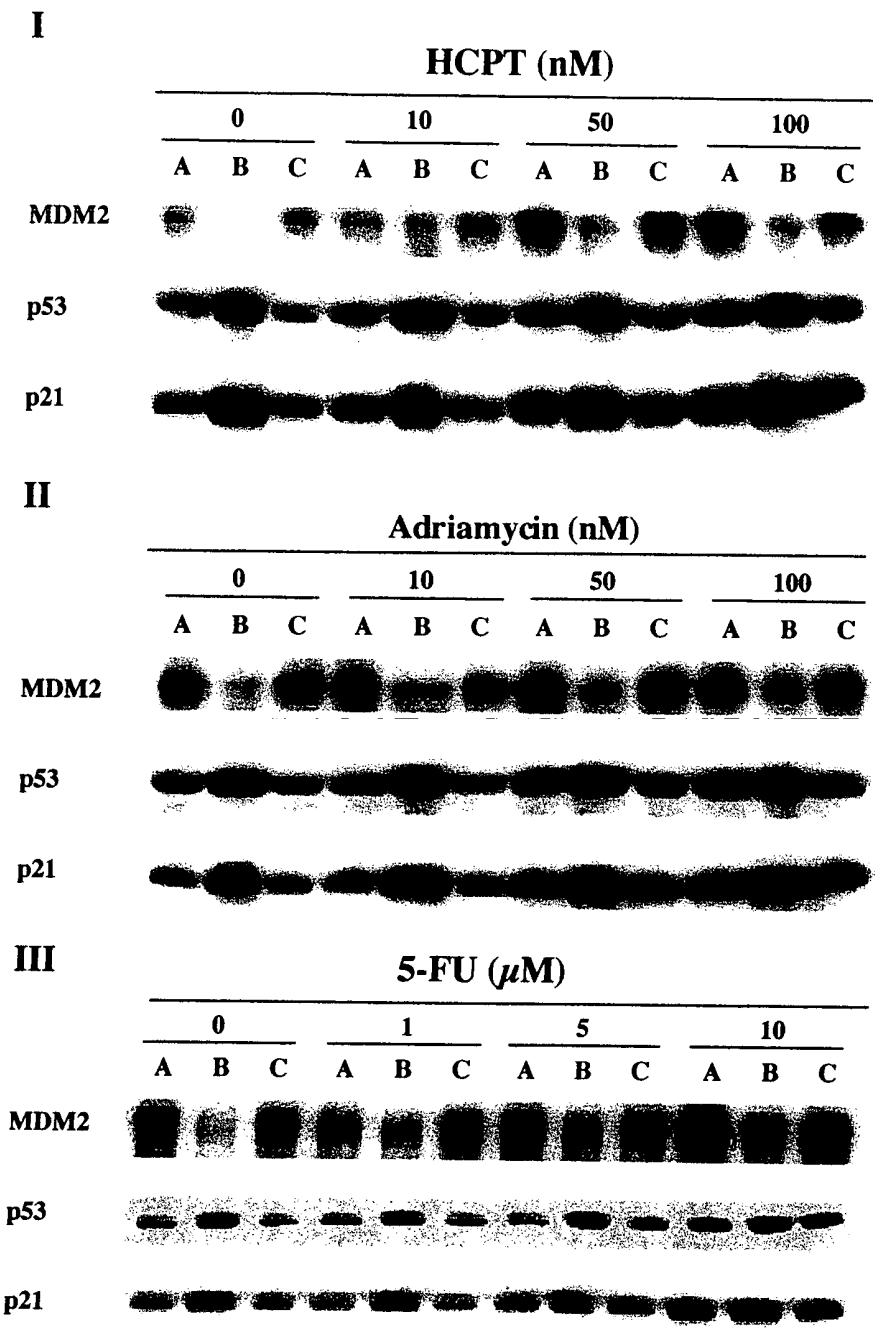

I
HCPT (nM)
0
10
50
100
A B C A B C A B C A B C
MDM2
p53
p21
II
Adriamycin (nM)
0
10
50
100
A B C A B C A B C A B C
MDM2
p53
p21
III
5-FU (μM)
0
1
5
10
A B C A B C A B C A B C
MDM2
p53
p21

Fig. 20

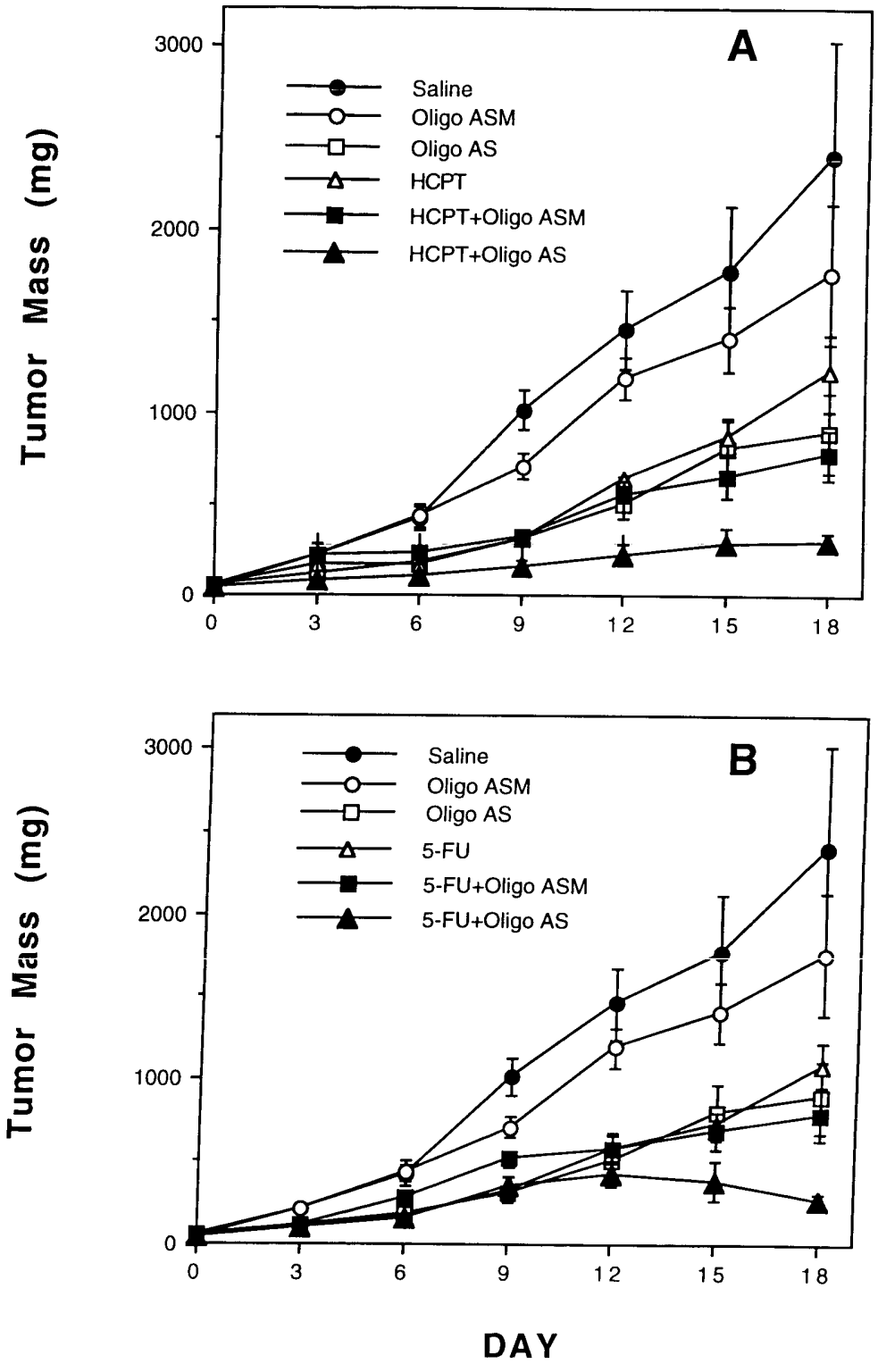

A
Saline
Oligo ASM
Oligo AS
HCPT
HCPT+Oligo ASM
HCPT+Oligo AS
Tumor Mass (mg)
3000
2000
1000
0
0
3
6
9
12
15
18
B
Saline
Oligo ASM
Oligo AS
5-FU
5-FU+Oligo ASM
5-FU+Oligo AS
Tumor Mass (mg)
3000
2000
1000
0
0
3
6
9
12
15
18
DAY

Fig. 21

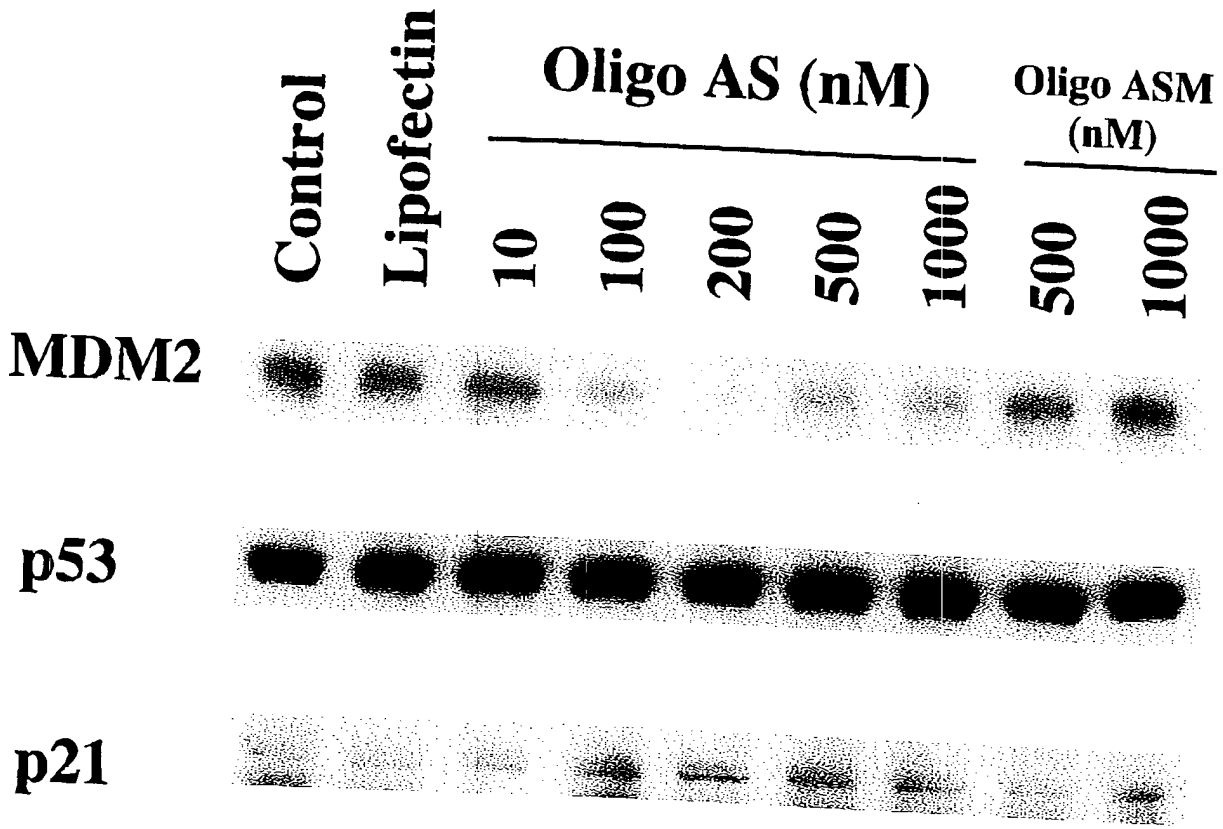


Fig. 22

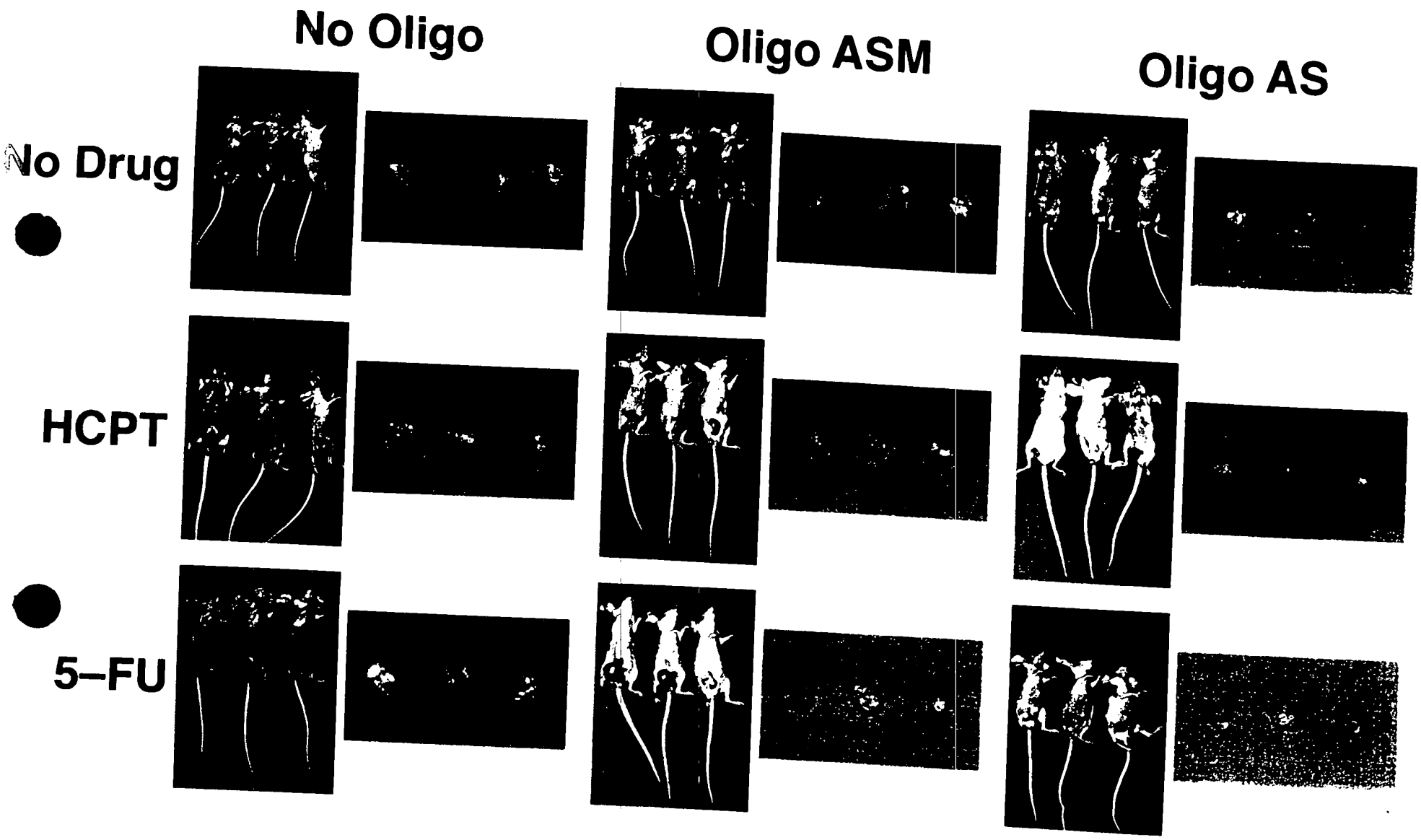


Fig. 23

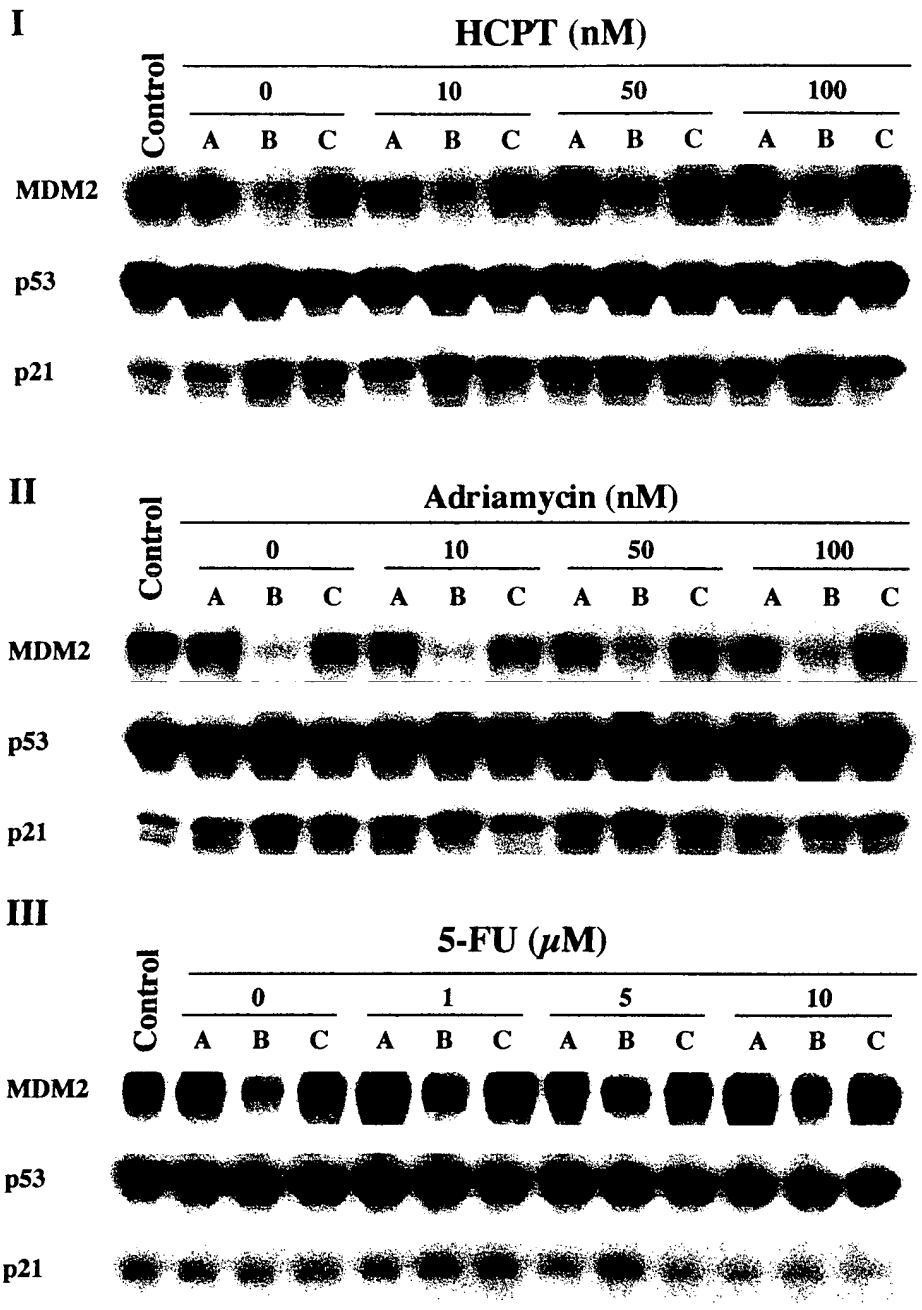

I
HCPT (nM)
Control
0
10
50
100
A B C A B C A B C A B C
MDM2
p53
p21
II
Adriamycin (nM)
Control
0
10
50
100
A B C A B C A B C A B C
MDM2
p53
p21
III
5-FU (μM)
Control
0
1
5
10
A B C A B C A B C A B C
MDM2
p53
p21

Fig. 24

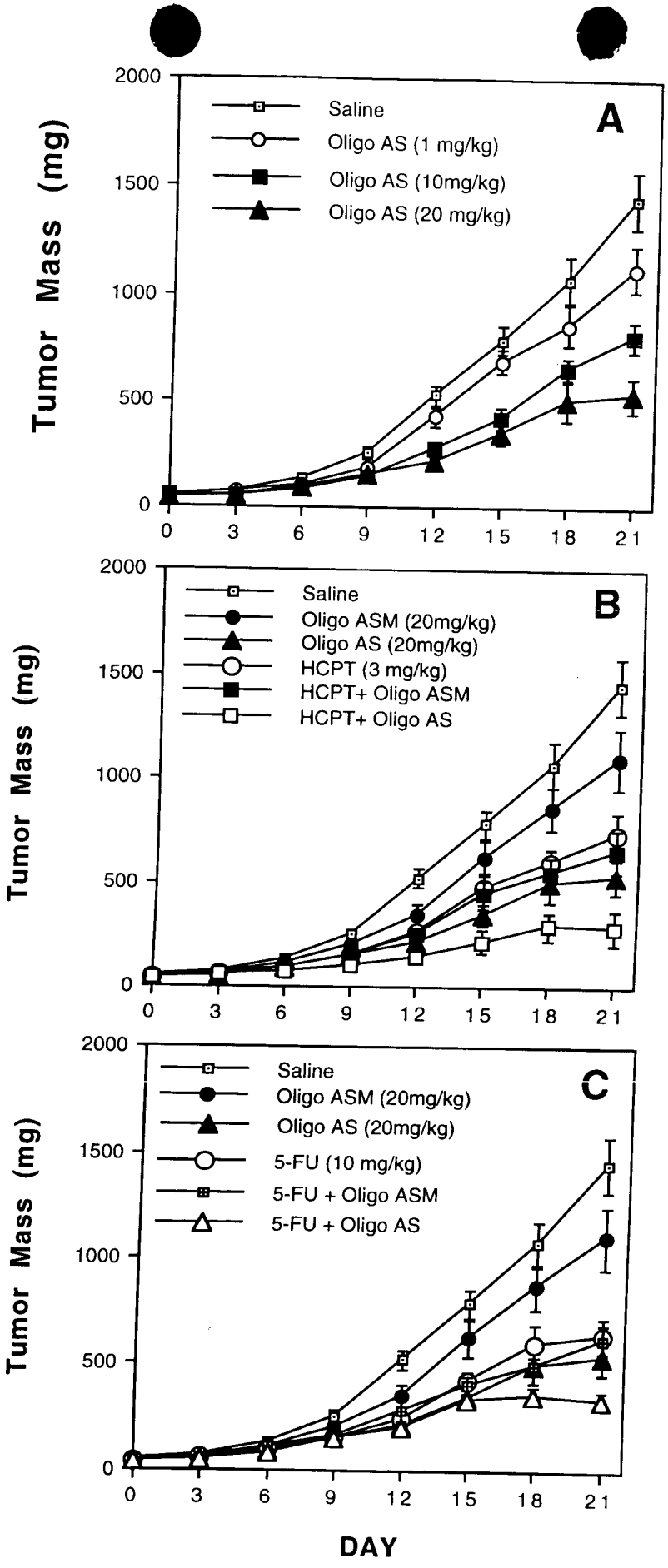

A
Saline
Oligo AS (1 mg/kg)
Oligo AS (10mg/kg)
Oligo AS (20 mg/kg)
Tumor Mass (mg)
2000
1500
1000
500
0
0 3 6 9 12 15 18 21
B
Saline
Oligo ASM (20mg/kg)
Oligo AS (20mg/kg)
HCPT (3 mg/kg)
HCPT+ Oligo ASM
HCPT+ Oligo AS
Tumor Mass (mg)
C
Saline
Oligo ASM (20mg/kg)
Oligo AS (20mg/kg)
5-FU (10 mg/kg)
5-FU + Oligo ASM
5-FU + Oligo AS
Tumor Mass (mg)
DAY

Fig. 25